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HOW SPARE RECEPTORS OPPOSE THE ACTION OF CERTAIN ANTAGONISTS

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Some non-competitive anatagonists appear to be more effective in opposing the actions of partial agonists than in opposing full agonists. The full agonist appears to be partially protected. A ternary complex model of drug action offers a possible explanation of such phenomena. We assume that a binary receptor-ligand complex must bind to an activator to form a ternary complex and the phenomenological activity is proportional to the ternary complex, not the binary complex, as is assumed in classical receptor-occupation theory. The binary complex of full agonists binds tightly to the activator while the binary complex of partial agonists binds less firmly to the activator. Noncompetitive antagonists which reduce the affinity of the binary complex to the activator will cause a reduction in maximum response and a rightward shift in the ED50 of partial agonists. By contrast the activity of a full agonist in a system possessing more receptor molecules than activator molecules (spare receptors) may be less altered when exposed to the antagonist. The maximum response

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of the full agonist may be only slightly reduced by the antagonist while the ED50 is shifted more noticeably to the right. Removing a large portion of the receptors may make the dose response curve to the full agonist resemble that of a partial agonist and induce full sensitivity to the antagonist. The qualitative features of this system resemble the blood pressure responses of pithed rats to α-1 adrenergic agonists in the presence of calcium blockers.

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Introduction

Recent work has called attention to calcium channel blockers which are noncompetitive antagonists to alpha-1 adrenergic drugs. While these antagonists are very effective against partial agonists, they are much less effective against full agonists (Ruffolo et al., 1984; Timmermans et al., 1985). The full agonist may be made more sensitive to the action of the antagonist by irreversible removal of some of the alpha-1 receptors, Ruffolo has described this effect as an instance of a general principle of pharmacology in which the presence of spare receptors confers, on a phenomenological response, resistance to the action of a noncompetitive antagonist (Ruffolo et al., 1984). In such cases, it is also observed that partial agonists are more susceptible to the antagonist. Indeed, the weaker the partial agonist, the greater the susceptibility to the antagonist (Ruffolo et al., 1984; Timmermans et al., 1985). It is our aim to show that a ternary complex model of drug action can offer an explanation of these phenomena and to discuss the manner in which the components of the ternary complex model may be manipulated to regulate alpha-1 adrenergic responsiveness.

The Ternary Complex Model

Figure 1 displays the reaction scheme for a simple ternary complex model. Receptor (R) can react with a ligand, (L). If the ligand is an agonist, the binary complex (RL), may react with an activator to form the three-molecule, ternary complex, (RLX). This ternary complex is the active form and it is assumed that phenomenological responses will be proportional to the concentration of ternary complex rather than to the concentration of the binary complex which is assumed to be inactive.

$$(L) + (R) \underset{}{\overset{K_1}{\rightleftharpoons}} (LR)$$

$$(LR) + (X) \underset{}{\overset{K_{1X}}{\rightleftharpoons}} (LRX)$$

This model is an extension of the classical receptor occupation model. The extended model includes partial agonism and spare receptors among the phenomena it can mimic. For this reason it appears a promising choice for modelling the effect of spare receptors on the performance of non-competitive antagonists. At equilibrium, the total concentration of the activator, X_T, is the sum of free activator X, and that bound to the ternary complex, T,

$$X_T = X + T. \quad [1]$$

The total concentration of receptor, R_T, is the sum of free concentration of receptor, R, the concentration of ternary complex, T, and the concentration of the binary complex, K_1RL.

$$R_T = R + T + K_1RL. \quad [2]$$

The binary complex concentration is the product of R, the ligand concentration L, and the receptor association constant, K_1. The ternary complex concentration at equilibrium is the product of the binary complex concentration, the free activating protein concentration, and the ternary complex association constant, K_{1X}.

$$T = (K_1RL)XK_{1X} \quad [3]$$

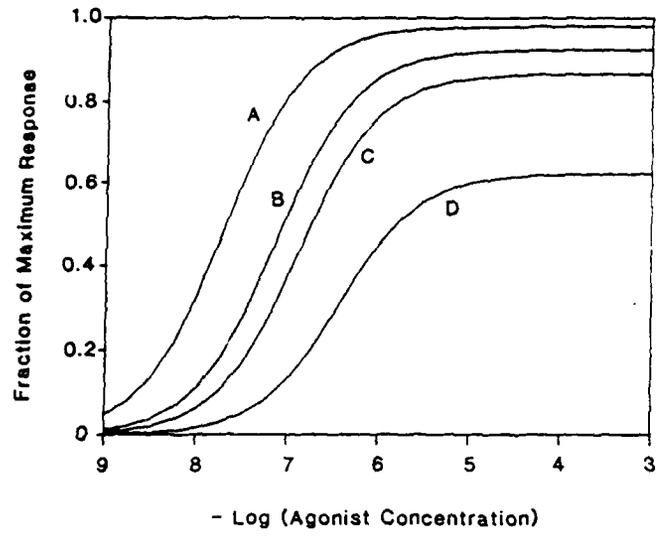
By solving Equation 1 for X and Equation 2 for R, and substituting these expressions into Equation 3, we obtain:

$$T = (R_T - T)\ (X_T - T)K_{1X}K_1L/(1 + K_1L). \quad [4]$$

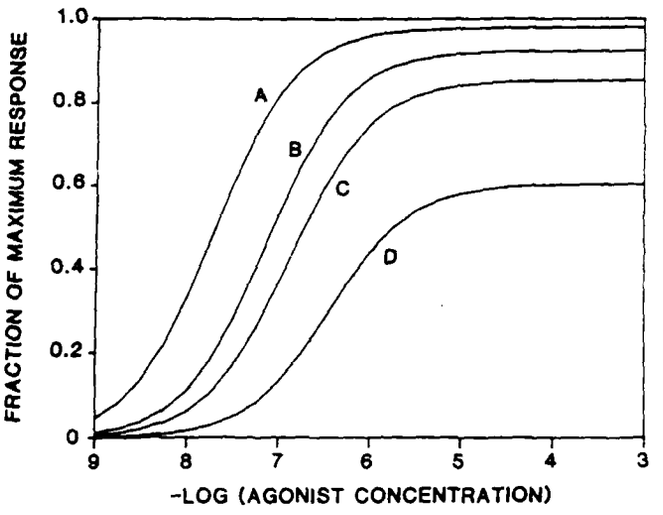
For our simulations, we have chosen $K_1 = 1 \times 10^{-6}\ M^{-1}$, and $X_T = 1$ functional unit. Total receptor concentration, R_T, and the ternary complex

association constant, K_{1X}, have been varied as needed to illustrate features of the ternary complex model. The value for K_1 corresponds to estimates in vascular smooth muscle for phenylephrine (Sastre et al., 1984). The choice for X_T is convenient since the values for T must now lie between 1 and 0 functional units. Since the phenomenological response is assumed proportional to T, we may choose the units of T as we wish. With X_T fixed at 1, R_T, necessarily in the same units, will express numerically the relative abundance of R_T. That is, $R_T = 100$ indicates a one-hundred fold excess of receptors over activating protein. Figure 2 shows several solutions of equation 4. The topmost curve, labeled A, represents the normal response to increasing concentrations of a full agonist. The curve labeled B represents the dose-response curve to the same agonist, but with K_{1X} depressed to one-fourth of its normal value by some non-competitive antagonist. Curve C represents a partial agonist whose maximum response is only 87% of the maximum possible response. Curve D shows a greatly depressed response to the partial agonist which is caused by decreasing K_{1X} to one-fourth the normal value for this partial agonist. In the presence of surplus receptors, a noncompetitive antagonist which decreases K_{1X} to one-fourth its normal value depresses the maximum response of a full agonist in our simulations by only 5%. The response of the partial agonist is depressed 30%. The antagonist shifts the dose response curves to the right even in circumstances in which the maximum response is not much affected. This general pattern has been reported to be characteristic of responses evoked by alpha-1 adrenergic agonists (Ruffolo et al., 1984; Timmermans et al., 1985). Figure 3 shows that

Partial Agonism and Antagonist Modulation



Receptor Excess and Antagonist Modulation



reducing the number of receptors can convert a response which is relatively insensitive to the noncompetitive antagonist into one which is more sensitive. Curve A, as before, shows the response to a full agonist while curve B shows the same response after a dose of noncompetitive antagonist which reduces K_{1X} to one-fourth of its normal value. Curve C represents the response to the full agonist after removal of 87% of the receptors leaving a 6.7 fold excess of receptors instead of the original 50-fold excess. The shape of the curve is very similar to that of the partial agonist (curve C) in figure 2. When the noncompetitive antagonist is added K_{1X} is reduced to one-fourth of its previous value and curve D demonstrates a striking sensitivity to the noncompetitive antagonist after most of the spare receptors have been removed.

DISCUSSION

When the receptors are present in great excess ($R_T >> X_T$), we may take R_T as an approximation to the free concentration, R, (Black et al., 1983) and T assumes the familiar hyperbolic independence on *L*.

$$T = T_M L/(ED50 + L). \quad [5]$$

The maximum response is,

$$T_M = K_{1X}R_T X_T/(1 + K_{1X}R_T). \quad [6]$$

The ligand concentration producing a response 50% of the maximum is.

$$ED50 = 1/K_1(1 + K_{1X}R_T). \quad [7]$$

Equation 7 indicates that decreases in K_{1X} or the concentration of receptors may be expected to lead to increases in ED50 just as shown in

Figures 2 and 3. The ED50 will, in general, be less than $1/K_1$. It will be much less if the receptor reserve is very large and $K_{1X}R_T$ correspondingly larger than one. As the receptor reserve diminishes, the ED50 approaches $1/K_1$ as has been reported experimentally (Ruffolo, 1982). Equation 6 shows us that the maximum is independent of K_1 and proportional to X_T. If the product $K_{1X}R_T$ is much larger than one, then T_M approaches X_T and the maximum will be rather insensitive to changes in R_T or K_{1X} as is shown in the small differences between curves A and B of figures 2 or 3. If $K_{1X}R_T$ is near one or smaller, then changes in R_T or K_{1X} will lead to large changes in the maximum. Even with $K_{1X}R_T$ = 6.7 (curve C, figure 2 or 3), a 76% reduction in K_{1X} can cause a 30% reduction in the maximum response as may be seen by comparing curve C with curve D in figure 2 or 3. T_M is a hyperbolic function of the product $K_{1X}R_T$. Since we suppose R_T to be the same no matter what the agonist used, partial agonists must be distinguished from full agonist by having smaller value of K_{1X} than a full agonist. Partial agonists having smaller values for K_{1X} will have smaller values for the product $K_{1X}R_T$ and hence smaller T_M's. T_M for a full agonist may be reduced to that of a partial agonist by removing enough receptors so that the product $K_{1X}R_T$ for the full agonist is reduced to the product for the partial agonist with normal R_T. Thus, in a sense, removal of receptors may convert a full agonist into a partial agonist.

Ruffolo et al., (1984) have suggested that certain non-competitive antagonist will be least effective against the response to full agonists in the presence of surplus receptors to the agonist. Such antagonists will be most effective against partial agonists or against

systems lacking a receptor surplus. They do not suggest a molecular basis for these observations. Beckeringh and coworkers (Berkeringh et al., 1984), suggested that alpha-1 agonists bind to two receptor subtypes, one calcium insensitive the other calcium sensitive. According to this view, full agonists rely predominantly on the calcium insensitive mechanism while a weak partial agonist relies mostly on the calcium sensitive pathway for expression of a response. It is not, however, necessary to invoke a dual mechanism of action. A single mechanism with characteristics of a ternary complex model may also display such complex behavior. De Lean and coworkers (De Lean et al., 1980), have suggested that Beta adrenergic responses may be governed by a ternary complex. Because binding of agonists to alpha-2 receptors was found to be sensitive to guanine nucleotide, it has been proposed that the α-2 receptor also forms a ternary complex (Hoffman et al., 1982). Later, it was shown that rat liver α-1 receptor binding of agonist was also sensitive to guanine nucleotide (Lynch et al., 1985). Such ternary complex models may describe both spare receptors and partial agonism. As we have now shown, a ternary complex model also predicts that full agonists may appear resistant to antagonists in the presence of a large receptor surplus while partial agonists will be relatively more sensitive. The sensitivity of α-1 adrenergic agents to calcium blockers would be explained if the binding of calcium to the activating protein increased the affinity of the activating protein for the binary complex, (increased K_{1X}). This appears to be true for the Beta melanotropin receptor (Salomon et al., 1986).

While a ternary complex model may serve as a qualitative explanation of some features of non-competitive antagonism, it is surely not the only model which might account for the data. The clearest studies delineating the relationship between receptor surplus and resistance to antagonism have been studies of blood pressure responses to alpha-1 agonists in pithed rats (Ruffolo et al., 1984; Timmermans et al., 1985). The antagonists used were calcium channel blockers. Such a system is surely complicated enough to admit many possible interpretations of the data, including, of course, the suggestion that separate calcium sensitive and calcium insensitive mechanisms of action exist for alpha-1 agonists.

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FIGURE LEGENDS

Figure 1 - Ternary Complex Formation. Receptor (R) and Ligand (L) react to form a binary complex (LR). The binary complex reacts with an activating protein (X) to form the ternary complex (LRX).

Figure 2 - Antagonist effects on full and partial agonists. $K_1 = 10^6$, $X_T = 1$, $R_T = 50$ for all curves. Curve A $K_{1X} = 1$. a full agonist. Curve B reduction of K_{1X} for the full agonist to ¼ normal value by antagonist. Curve C $K_{1X} = .133$. a partial agonist. Curve D reduction of the partial agonist K_{1X} to ¼ of .133 by an antagonist.

Figure 3 - Sensitization to antagonist by removal of receptors. $K_1 = 10^6$, $X_T = 1$ for all curves. Curve A $R_T = 50$, $K_{1X} = 1$, normal full agonist. Curve B $R_T = 50$, $K_{1X} = .25$ response to full agonist after treatment with antagonist, curve C $R_T = 6.7$, $K_{1X} = 1$, 87% of receptors inactivated, curve D $R_T = 6.7$, $K_{1X} = .25$, response to full agonist after removal of receptor and exposure to antagonist.